DETERMINATION OF ELEMENTAL UPTAKE RATES DURING THE EARLY LIFE STAGES OF WALLEYE (*SANDER VITREUS*)

SENIOR HONORS THESIS

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By

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Chapter I: Determining if larval walleye uptake rates are rapid enough to establish a natal site signal

Introduction

In Lake Erie, walleye (*Sander vitreus*) represent the largest and most important sport fishery. To effectively manage this fishery, it is important to understand the early life history of walleye and the environments in which they spend these critical life stages. In the western basin of Lake Erie, walleye have been shown to spawn in the Maumee River, Sandusky River, and the open reefs of Lake Erie (Wang et al. 2007). Understanding how different spawning stocks are contributing to the population in Lake Erie is an important step in managing the Lake Erie walleye population.

Fish otoliths, or ear stones, are commonly used tool in fisheries research, helping researchers and managers to obtain a variety of data, including dates of hatching, growth rates, age, and early life history of fish. Although the function of the otolith is balance and hearing (Campana and Neilson 1985, Popper et al. 2005), otoliths are useful to fishery managers because throughout their life, fish actively take up trace elements from the water around them. Elements incorporated into the calcium carbonate matrix of the otolith will not be reabsorbed, as the otoliths are metabolically inert (Fowler et al. 1995, Campana and Neilson 1985). Therefore, otoliths are reliable indicators of environmental conditions experienced throughout a fish's lifetime.

As fish take up elements daily, when the otolith is extracted and processed, researchers are able to compare the elemental concentration in the otolith with that of surrounding waters to determine where a fish hatched and the different environmental conditions experienced since hatch. When using otoliths of adult fish, otolith

microchemistry can be used to track migration patterns (Campana 2007, Daverat et. al 2005, McCulloch et. al 2005) and determine stock contributions to a fishery (Jonsdottir 2006, 2007, Fowler et. al 2005). However, most of the research done using otoliths has been conducted in marine environments and previous research has demonstrated that salinity level can influence elemental uptake (Elsdon and Gillanders 2005). Therefore, in order to apply this technique to freshwater species, these experiments must be repeated.

Although previous research has determined that fish lay down elemental signatures daily, there is a lag between elemental exposure and occurrence in the otolith at concentrations great enough to detect. Such an uptake lag may lead to ambiguous or misleading results when assigning individuals to specific habitats. Larval walleye, carried by currents, are able to move out of spawning streams within a few days (Mion et al. 1998). Therefore, it also important to determine if a larval walleye is able to establish a site signal while still in the spawning stream.

This experiment was designed to determine at what point in the early life history of the walleye (*Sander vitreus*) a chemical signal, specifically that of strontium, is first recorded in the otolith and whether uptake rates are rapid enough to record a natal site signal. This experiment will also demonstrate how environmental conditions influence otolith elemental concentration in larval walleye otoliths, as the experimental design uses two different temperatures and three elemental concentrations. In determining if larval walleye otolith uptake rates are rapid enough to record a natal site signal, the effectiveness of using the otolith to assign a natal origin will also be assessed.

Materials and Methods

Experimental Design

In cooperation with the Ohio Division of Wildlife, we used electrofishing to collect adult spawning walleye (5 males and 5 females) from the Maumee River near Perrysburg, Ohio on April 8th, 2008. Adult walleye were measured for length and weight and their gonads were stripped for spawning, using procedures detailed by Rinchard et. al 2005. Walleye were selected to span a range of sizes. Eggs and milt were stored in separate polypropylene containers and placed into a cooler.

Eggs and milt were transported back to the Aquaculture Laboratory at the Ohio State University for fertilization. Eggs were fertilized using procedures modified from Rinchard et al. 2005. Eggs were taken from their containers and combined into a 5-gal bucket. Milt was then added. All of the eggs and milt were mixed together in an attempt to randomize the effects of parental condition on otolith microchemistry. Eggs and milt were mixed together for 5 min using a plastic spatula. Tannic acid, at a concentration of 400 mgL^{-1}, was added until the eggs and milt were completely submerged. Fertilized eggs were then stirred again for five minutes. After five minutes, the fertilized eggs were rinsed with water three times.

After all the tannic acid was removed, eggs were treated with iodine to ensure that they did not have Viral Hemorrhagic Septicemia. Iodine was added until the fertilized eggs were completely submerged. Eggs were then stirred continuously for 30 min. During this time, fertilized eggs were transported to the Aquatic Ecology Laboratory at the Ohio State University. After 30 min, eggs were rinsed three times with water. Immediately after being rinsed, eggs were transferred to McDonald-style jars connected

to 100 L tanks. Tanks were filled with water from a common initial source (Columbus city water, Sr concentration 232 ug/L) and treatments (300 ug/L, 900 ug/L, 1500 ug/L Sr) were established by spiking water with strontium chloride and controlling the temperature. Eggs for each elemental treatment were incubated in closed, recirculating systems, with two replicates for each elemental treatment, with three McDonald jars per system. All systems were maintained at approximately the same temperature (average 13.14°C) while the eggs were incubating. Dissolved oxygen and temperature readings were taken every day for each system. To ensure that Sr concentrations were being properly maintained, water samples were taken on Monday, Wednesday, and Friday. Dead eggs were siphoned out of the McDonald jars daily and discarded to prevent growth of fungus.

The first larvae hatched on April 16th, 2008, 9 days after fertilization (D=0). On April 17th, 2008, 10 days after fertilization, air stones were inserted into each McDonald jar for 20 minutes to induce hatching. On the same day, larvae were transferred randomly into experimental tanks. The three elemental concentrations were crossed with two temperatures. These temperatures and elemental concentrations were chosen in order to represent a range of conditions found in Lake Erie and its tributaries. For example, 300 ug/L of Sr is comparable to the conditions found in the open water of Lake Erie, while 900 ug/L of Sr is comparable to the Sr concentration found in the Maumee River and 1500 ug/L of Sr is the concentration of Sr that can be found in the Sandusky River. For half the larvae, the temperature was lowered to 8°C, while the remaining larvae were maintained at 13°C. There were four replicates of each elemental treatment at each temperature (3 elemental treatments x 2 temperatures x 4 replicates = 24 total tanks).

Dissolved oxygen and temperature readings were taken every day for each tank. Water in the tanks was changed on Monday, Wednesday, and Friday. Water samples were taken immediately after each water change.

Egg samples were taken 4 and 2 days before hatch and on the day of hatch (*D*= -4, -2, 0). Larval samples were taken every other day, beginning with day of hatch, for twenty days. For the 13°C fish, the experiment was stopped at 20-d post-hatch. However, for the 8°C fish the experiment was continued for 32 days post-hatch so that the experiment ended at the same number of degree days as the 13°C treatment.

Otolith Removal and Analysis

Otoliths were removed from 20-d post-hatch fish (See chapter 2 for specific handling procedures) and the Sr:Ca ratio was measured with laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) performed by Ohio State's Trace Element Research Laboratory. A New Wave Research UP-193HE laser ablation system consisting of a 193 nm excimer laser with beam homogenizing optics was used with a ThermoFinnigan Element 2 Inductively Coupled Plasma Sector Field Mass Spectrometer.

To compare the amount of strontium in the water with the amount in the otolith, a partition coefficient was calculated using the following equation modified from Bath et al. 2006. The Sr:Ca ratio of the water was determined using SO-ICPMS measurements of water samples.

Results

A two-way ANOVA test was performed to determine the effects of elemental treatment, temperature, and their interaction on larval otolith elemental uptake. The 20-d post hatch larvae showed a treatment related strontium signal, $p < 0.0001$ (Table 1). Temperature was determined to not have an effect on the otolith uptake rates of larval walleye ($p = 0.98$). However, there was a significant ($p = 0.003$) interaction between the two.

Factor **Dependent variable**	**d.f. (model, error)**	***F-ratio***	**P**
Elemental treatment	2,58	41.9	< 0.0001
Temperature	1,58	0	0.98
Interaction	2,58	6.37	0.003

Table 1. Two-way ANOVA, showing the effects of elemental treatment, temperature, and their interaction.

Otolith Sr:Ca (mmol:mol) increased with increasing Sr:Ca in the water (ANOVA, elemental treatment $F_{2,58} = 41.0$, $p < 0.0001$; Table 1, Fig. 1). Temperature did not have a significant effect on otolith Sr:Ca (ANOVA, temperature treatment $F_{1,58} = 0$, $p = 0.98$; Table 1, Fig. 1), the interaction between temperature and elemental concentration was significant (ANOVA, interaction $F_{2,58} = 6.37$, $p = 0.003$; Table 1, Fig. 1). For both the low and high treatments, otolith Sr:Ca was higher at 13ºC than at 8 ºC. However, this

trend did not hold for the control treatment group. For the control treatment, otolith Sr:Ca was higher at 8 ºC than it at 13ºC.

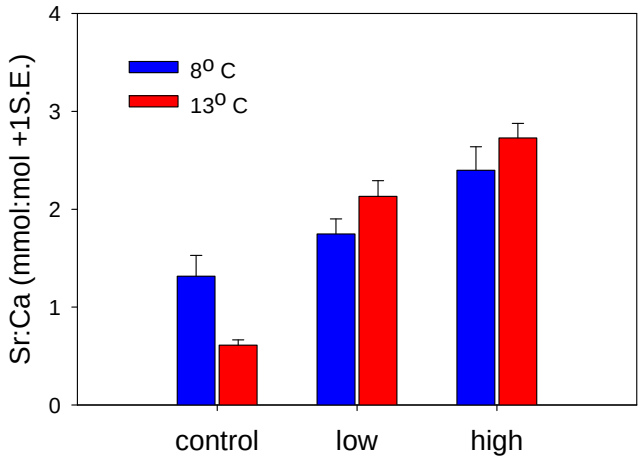

Figure 1. Sr:Ca (mmol:mol) plotted by treatment group. Sr:Ca is higher at 13ºC than at 8ºC for both the low and high treatments, but is lower for the control treatment.

A partition coefficient was calculated to determine the proportion of strontium in the water that was incorporated into the otolith (Figure 2). The trace metal composition of otoliths ($[Sr/Ca]_{otolith}$) can be related to that of the water ($[Sr/Ca_{H20}]$) through a partition coefficient (D_{Sr}) (Bath et al. 2000).

$$\left[\frac{Sr}{Ca}\right]_{otolith} = D_{Sr}\left[\frac{Sr}{Ca}\right]_{H_2O}$$

A partition coefficient of 1 would represent a “perfect” partition coefficient (ie. the concentration of Sr per mole Ca in the otolith is equal to the concentration of Sr per mole Ca in the water). The partition coefficient was similar at both temperatures for both the low and high treatments. For the control treatment, the partition coefficient for the 13°C

treatment was similar to the partition coefficients for the low and high treatments. However, there was much more variation in the partition coefficient for the control treatment at 8°C.

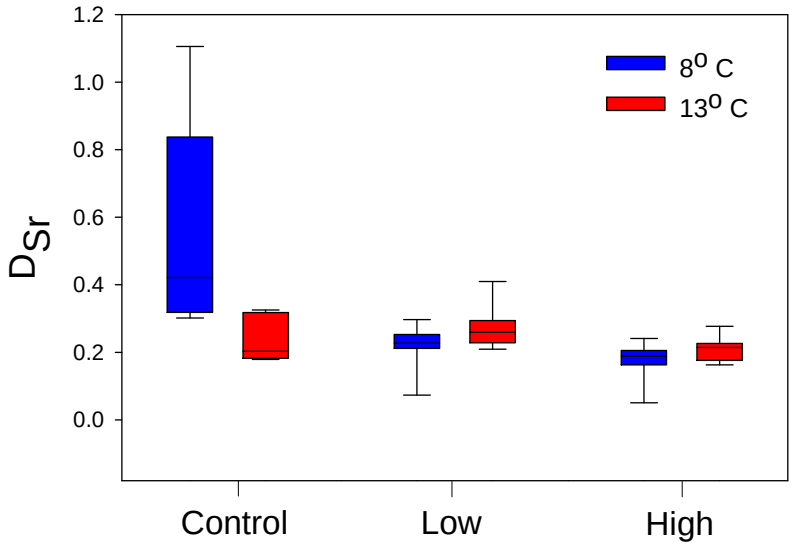

Figure 2. Box plots of partition coefficient plotted by treatment. The median is represented by the black line. The colored portion of the box represents the 25th to 75th percentile. The low end of the whisker represents the 10th percentile, while the upper end represents the 90th percentile.

Discussion

According to the two-way ANOVA, by 20-d post-hatch, larval otoliths showed a treatment-related strontium signal (Table 1). In this experiment, temperature did not appear to have an effect on larval otolith uptake rates. However, the significant interaction between the two indicates that, although there may not be an overall temperature effect, within each temperature group, temperature did have an effect. This effect is evident when looking at the difference in the trend that the 8°C control treatment

fish exhibit. The result obtained in this study, that at very low temperatures and very low elemental concentrations otolith elemental uptake rates follow patterns that are different from uptake rates at warmer temperatures and higher elemental concentrations, is similar to previous results obtained at the Aquatic Ecology Laboratory.

The Sr:Ca ratio was determined to be higher at 13°C than at 8°C for both the low and high treatments but this trend does not hold for the control treatment. For the control treatment, the Sr:Ca ratio was higher at 8°C than at 13°C. When the partition coefficient was calculated, the control treatment at 8°C also demonstrated behavior different from the trend. This indicates that at 20-d post-hatch, walleye from the Maumee River can be distinguished from walleye spawned in the Sandusky River. However, due to the high variability in the partition coefficent at low temperatures and low elemental concentrations, walleye that were spawned on the open water reefs of Lake Erie might be more difficult to distinguish.

Although this research has established that at 20 days post-hatch walleye larvae otoliths display a treatment related strontium signal, analyses must be continued to determine exactly what day a strontium signal can be detected in the otolith. Larval walleye travel downstream quickly and can be deposited by river discharge into the open waters of Lake Erie in a matter of days. Therefore, it is important to determine if walleye larvae establish a site signal prior to this.

Analyses are currently being continued to determine at exactly what day a larval walleye lays down a treatment related strontium signal. When we attempted to extract the otoliths from younger walleye, the otoliths were difficult to locate and extract because of their small size (typically less than 20 um across). Therefore, we have begun using the

combination of a novel bleach technique (Chittaro et al. 2006), in which the entire fish is dissolved and the undissolved otoliths are filtered from the solution, and solution-based inductively coupled plasma mass spectrometry (Ludsin et al. 2006).

Chapter II: Comparing processing methods for analysis using LA-ICPMS

Introduction

When preparing fish otoliths for microchemical analysis, sonication has become the normal procedure for cleaning the otolith. Sonication involves placing the otolith into a machine called a sonicator that uses ultrasonic waves to clean contamination off the otolith. Many of these sonication procedures are complex and require several steps. However, when smaller otoliths (16 – 20 um) are being used, such as in this experiment, sonication proves both time-consuming and risky, as otoliths are easily broken or lost. An experiment was performed to determine if sonication is necessary in preparing otoliths for microchemical analysis.

Materials and Methods

Larvae were reared at three strontium concentrations (300 ug/l, 900 ug/l, 1500 ug/l**)** at two different temperatures 8°C and 13°C, as described in Chapter 1. The larvae that were used for this experiment were 20-d post-hatch. Total length of larvae was recorded before otolith extraction. All of the following procedures were carried out in a class-100 laminar flow hood with non-metallic acid-washed instruments. Both saggital otoliths were extracted using glass probes. One otolith was randomly chosen to go through the sonication process, while the other otolith did not. Otoliths chosen for the non-sonication treatment were rinsed in one drop of Super-Q water, which has been demineralized and deionized, and then mounted using double-sided tape (Scotch ® Permanent Double Sided Tape) on a petrographic microscope slide.

The otoliths that were to be sonicated were handled according to procedures modified from Campana et al. 2000. Once extracted, the otolith was placed into a drop of

Super-Q water to rinse excess tissue from the otolith. With a clean probe, the otolith was then transferred from the water droplet into another drop of Super-Q water in a polystyrene petri dish. Otoliths were then sonicated for 5 minutes at level 3 power setting. Following sonication, the otolith was removed from the petri dish and rinsed three times in a drop of Super-Q water. The otolith was then mounted on the petrographic slide.

Otoliths were processed using laser ablation – inductively coupled plasma mass spectrometry. A New Wave Research UP-193HE laser ablation system consisting of a 193 nm excimer laser with beam homogenizing optics was used with a ThermoFinnigan Element 2 Inductively Coupled Plasma Sector Field Mass Spectrometer. For analysis, a three spot average, with spots near the edge of the otolith, with a laser pulse energy of 1 mJ and a pulse frequency of 10 Hz was used. Immediately prior to analysis, each otolith was shot with a single, low-powered laser pulse (around 20 uJ), covering the entire otolith to remove any particles on the surface.

Results

Using a two-way ANOVA, comparing the effects of sonication and elemental treatment, the otolith Sr concentration did not differ between sonication and non-sonication otolith treatments ($p=0.85$). Otolith Sr increased with increasing Sr in the water ($p<0.001$). The interaction between sonication treatment and elemental treatment was not significant ($p=0.97$).

When the Sr concentration (ppm) of sonicated otoliths is plotted against the Sr concentration (ppm) of non-sonicated otoliths, the Sr concentrations of pairs of otoliths falls on a one-to-one line (Figure 3).

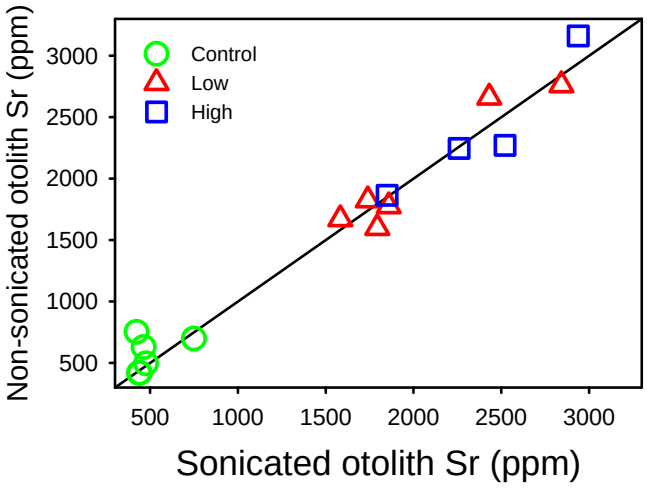

Figure 3. Plot of Sr (ppm) of sonicated vs. non-sonicated otoliths. R^2*=0.8159.*

Discussion

As the Sr concentrations of otolith pairs used in the sonication treatments falls close to the one-to-one line, it can be concluded that sonication is not needed when using a low-powered laser pulse prior to chemical analysis. As the sonication step is time consuming and many otoliths are lost during the process, not using sonication when preparing otoliths for microchemical analysis will save a significant amount of time. Without sonication, otolith processing will be much more efficient, saving approximately twenty minutes per otolith and significantly reducing the number of otoliths that are lost and broken.

Literature Cited

Bath, G.E., S.R. Thorrold, C.M. Jones, S.E. Campana, J.W. McLaren, and J.W. Lam. 2000. Sr and Ba uptake in otoliths of marine fish. Geochimica et Cosmochimica Acta 64: 1705-1714.

Campana, S.E. and J.D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42: 1014-1032.

Campana, S.E. A. Valentin, D.M. Sevigny. Tracking seasonal migrations of redfish (Sebastes spp.) in and around the Gulf of St. Lawrence using otolith elemental fingerprints. Canadian Journal of Fisheries and Aquatic Sciences 64: 6-18.

Campana, S.E., G.A. Chouinard, J.M. Hanson. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fisheries Research 46: 343-357.

Chittaro, P.M., J. Gagnon, B.J. Fryer. 2006. In situ experiment of ontogenetic variability in the otolith chemistry of *Stegastes partitus*. Marine Biology 149: 1227-1235.

Daverat, F., J. Tomas, and M. Lahaye. Tracking continental habitat shifts of eels using otolith Sr/Ca ratios: validation and application to the coastal, estuarine and riverine eels of the Gironde-Garonne-Dordogne watershed. Marine and Freshwater Research 56: 619-627.

Elsdon, T.S. and B.M. Gillanders. 2005. Consistency of patterns between laboratory experiments and field collected fish in otolith chemistry: an example and applications for salinity reconstructions. Marine and Freshwater Research 56: 609-617.

Fowler, A.J., S.E. Campana, and C.M. Jones. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition using laser-ablation ICPMS. Canadian Journal of Fisheries and Aquatic Sciences 52: 1431-1441.

Fowler, A.J., G.A. Begg, and S.E. Campana. 2005. Otolith research and application: current directions in innovation and implementation. Marine and Freshwater Research 56: 477-483.

Jonsdottir, I.G., S.E. Campana, and G. Marteinsdottir. 2006. Stock structure of Icelandic cod *Gadus morhua* L. based on otolith chemistry. Journal of Fish Biology 69: 136-150.

Jonsdottir, I.G., G. Marteinsdottir, S.E. Campana. 2007. Contribution of different spawning components to the mixed stock fishery for cod in Icelandic waters. ICES Journal of Marine Science 64: 1749-1759.

Ludsin, S.A., B.J. Fryer, J.E. Gagnon. 2006. Comparison of solution-based versus laser ablation inductively coupled plasma mass spectrometry for analysis of larval fish

otolith microelemental composition. Transactions of the American Fisheries Society 135: 218-231.

McCulloch, M.T., H.M. Patterson, and M.J. Kingsford. Resolution of the early life history of a reef fish using otolith chemistry. Coral Reefs 24: 222-229.

Mion, J.B., R.A. Stein, and E.A. Marschall. 1998. River discharge drives survival of larval walleye. Ecological Applications 8: 88-103.

Popper A.N., J. Ramcharitar, and S.E. Campana. Why otoliths? Insights from inner ear physiology and fisheries biology. Marine and Freshwater Research 56: 497-504.

Rinchard, J., K. Dabrowski, J.J. Van Tassell. 2005. Optimization of fertilization success in *Sander vitreus* is influenced by the sperm:egg ratio and ova storage. Journal of Fish Biology 67: 1157-1161.

Wang, H.Y., E.S. Rutherford, and H.A. Cook. 2007. Movement of walleyes in lakes Erie and St. Clair inferred from tag return and fisheries data. Transactions of the American Fisheries Society 136: 539-551.